Neurodevelopment, Autism, and Mercury: Biomarkers and Epidemiologic Approaches

University of California Davis,
M.I.N.D. Institute

Outline

- Outcomes
- Literature on:
  - Hg & Neurodevelopment
  - Hg & Autism: vaccines
  - Hg & Autism: non-vaccine sources
- UC Davis CCEH: *The CHARGE Study*
  - Goals, methods & results for Blood Hg
- Discussion & Next steps

Outcomes

- Developmental delay or deficits
- Mental or Cognitive:
  - Language
  - Memory
  - Spatial
  - Executive function
- Neuromuscular
- Sensory deficits
- Social: autism

What is Autism?

Pervasive developmental disorder defined as characterized by three behavioral domains:

- Deficits in social interaction
- Communication: Language impairment/delay or unusual speech patterns
- Repetitive behaviors and/or restricted interests

Facts about Autism

- Male:female ratio is 4
- Current reliable prevalence estimates: 1 in 150 (=60-70 per 10,000)
- Strong genetic component
  - 60-90% concordance: monozygotic twins
- Multifactorial
- Wide variation in severity, trajectory
- Historically attributed to 'bad parenting'
- Now known to have a neurobiologic basis: aberrant brain development
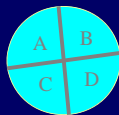
Neural Substrate of Autism

- Anatomical, electrophysiologic, MRI
- fMRI: esp face processing
- Highly diffuse throughout the brain:
  - Cerebellum
  - Hippocampus
  - Amygdala
  - Cerebral cortex

$\Rightarrow$ early insult

- Purkinje cell loss

Mercury and Neurodevelopment

Mercury: food contamination episodes

- 1953-1961 Minimata, Japan, chronic
- 1970's Iraq
  - *in utero* exposure ~ mental retardation, physical impairments, seizures
  - autopsies showed abnormal neuronal migration, disorganized cerebral cortex

Mercury and Neurodevelopment

Chronic Hg intake via fish consumption - (lower levels)

- Faroe Islands (n~900) Grandjean et al 1997, Debes 2006

prenatal exposure: maternal hair, cord blood, cord tissue
  - at 7 years: deficits in language, attention, memory, visuospatial domains
  - at 14 years: deficits in motor, attention, verbal
- Seychelles Islands   Davidson et al 2005
  - at 5.5 years: no deficits in language, visual-motor integration, various cognitive domains
- Project Viva (n=135)   Oken et al 2006

maternal hair mercury
  - at 6 months : deficit in visual recognition memory
 - able to separate beneficial effects of fish consumption from harmful effects of mercury

RCT of dental amalgams in school-aged: no effect

Mercury and Autism: Vaccines

- Controversy: thimerasol as preservative breaks down to ethyl Hg and thiosalicylate
- Polarization "Autism: a novel form of mercury poisoning" (Bernard et al)
- Removed from most childhood vaccines in 2001/02
- Numerous ecologic studies using before/after comparisons
- Few studies with individual-level data: Verstraeten et al of CDC…Vaccine Safety Datalink Rh- and thimerasol-containing rhogam
- Clarify: issue of MMR is unrelated

Epidemiologic Considerations
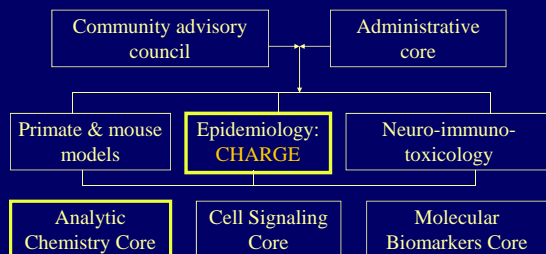
Hviid et al 2003
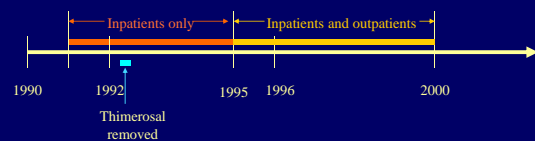
Sources of Hg:

- Combustion of fossil fuels
- Consumption of seafood or ocean or freshwater fish
- Erosion of dental amalgams
- Occupational exposure from mining
- Direct contact from damaged mercury thermometers, blood pressure cuffs, barometers, incandescent lights, or batteries.
- Dermal absorption from skin-lightening cremes
- Use of nasal sprays, ear wax removal products, contact lens solutions
- Vaccines

Studies of (nonvaccine) Hg & Autism

- Palmer et al (2006): TRS emissions inventory, Hg only & autism rates by school district
  - Special education availability - confounder
- Windham et al (2006): HAP model for multiple ambient pollutants
  - Used model-based estimated exposure for a different year (1996) than the births (1994)
- Both reported associations with autism
- Both were ecological
- *Think about timing!!*

UC Davis Center for Children's Environmental Health (CCEH)

- Community advisory council
- Administrative core
- Primate & mouse models
- Epidemiology: CHARGE
- Neuro-immuno-toxicology
- Analytic Chemistry Core
- Cell Signaling Core
- Molecular Biomarkers Core

Hg in the *CHARGE* Study

*CH*ildhood *A*utism *R*isk from *G*enetics and the *E*nvironment

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Goals of the CHARGE Study

To identify causes and contributing factors for childhood autism:
- Genetic susceptibility factors
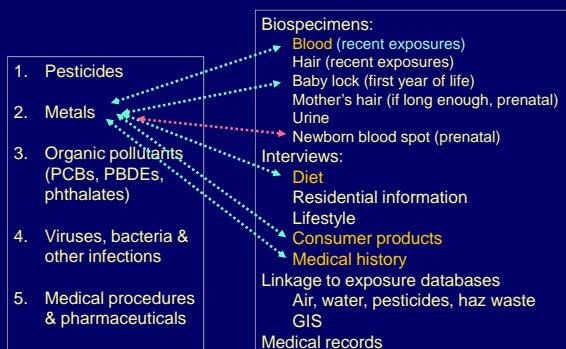- Environmental exposures
- Interplay of the two

To determine mechanisms of susceptibility for childhood autism
- Immunologic
- Genetic/Genomic
- Metabolic/metabolomic

Mechanisms for Xenobiotics

- Direct action on neural tissue during:
  - Differentiation
  - Migration
  - Dendritic branching/pruning
  - Synaptogenesis
- Effects on genes that regulate CNS development: neuroligin 4
- Perturbation of immune signaling/inflammation via neurotransmitters &/or cytokines
- Endocrine disruption: sex steroids/thyroid hormones

Environmental Exposures

1. Pesticides
2. Metals
3. Organic pollutants (PCBs, PBDEs, phthalates)
4. Viruses, bacteria & other infections
5. Medical procedures & pharmaceuticals

Biospecimens:
- Blood (recent exposures)
- Hair (recent exposures)
- Baby lock (first year of life)
- Mother's hair (if long enough, prenatal)
- Urine
- Newborn blood spot (prenatal)

Interviews:
- Diet
- Residential information
- Lifestyle
- Consumer products
- Medical history

Linkage to exposure databases
- Air, water, pesticides, haz waste
- GIS

Medical records

METHODS

The CHARGE Study Design

Case-control design…three groups:

- Children with autism
- Children with developmental delay
- Children drawn using probability sampling from the general population of births

Eligibility Criteria in the CHARGE Study

Children :

- 24-60 months of age
- born in California
- parents speak English and/or Spanish
- child living with at least one biologic parent
- residing in catchment area

CHARGE Study Recruitment Protocol:

Autism or Developmental Delay

- Recruited from Department of Developmental Services (DDS)

Population-based Controls

- State birth files, frequency matched to autism group by age, gender & Regional Center

The CHARGE Study Clinical Protocol

Confirmation of diagnosis:

- ADI and ADOS (autism cases only)
- Social Communication Questionnaire (all others)

Assessment of cognitive development (all children)

- Mullens Scales of Early Learning
- Vineland Adaptive Behavior Scales
- medical exam

Parent forms

Medical records obtained

## The CHARGE Study Clinical Protocol

- Parents interviewed:
  - Family History Interview
  - Exposures, events, activities
    covering prenatal, early childhood periods
- Specimens:
  - blood
  - urine
  - hair
    …from index child, parents & siblings
- newborn blood spots from CDPH GDB

Measurement of Blood Hg

- Blood collected by venipuncture into metal-free tubes
- Analyzed for metals using ICP-MS: Inductively Coupled Plasma Mass Spectrometry
- Detection limit: 1 pg/ml in blood
- Blood samples thawed, diluted in .005% solution of metal-free surfactant into sterilized double de-ionized H_2O with nitric acid.
- QC: cleaning exterior & interior; calibration every 10th sample
- In every batch of 30-40, NY state proficiency standards at low, medium and high levels

Hair Hg measurement - time course

ICP-MS served by Laser Ablation system with digital imaging
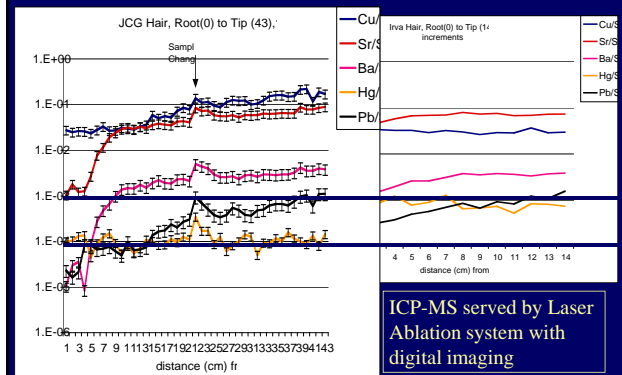
Statistical Analysis

- Duplicate entry into online system
- Immediate flag of discrepancies
- Histograms, log transformation of Hg

Prediction model for blood Hg concentration:
- Multiple linear regression adjusting for recent:
  - Fish consumption
  - Dental amalgams
  - Use of nasal sprays, ear drops

Also excluded children who had been chelated, and adjusted for differential participation by SES

RESULTS

Participation:

70% among Autism group

50% among General Population controls

Blood specimens analyzed for Hg:

271 AU (on ADOS or ADI)

61 DD

144 GP

Confirmation of diagnoses:

63% meet criteria for AD on both ADOS+ADI

98% meet criteria for AD or ASD on at least one

Log Hg and Covariates by Case Status

	Autism/ASD (N=271)			GP Typical (N=144)			Delayed (N=61)		
	Mean	STD	%>0	Mean	STD	%>0	Mean	STD	%>0
Log Hg	-1.71	1.54		-1.25	1.38		-1.68	1.54	
Servings tuna	0.11	0.31	16%	0.44	0.69	42%	0.39	0.62	42%
Servings ocean fish	0.29	0.50	37%	0.53	0.75	55%	0.39	0.59	44%
Servings freshwater fish	0.06	0.28	7%	0.20	0.51	19%	0.18	0.48	17%
Frequency nasal spray or ear wax removal	0.39	0.94	19%	0.22	0.63	13%	0.51	1.14	22%
Number amalgams x Chew or grind teeth	0.10	0.59	4%	0.18	0.87	5%	0.52	1.80	10%
Thimerosal dose*	0.005	0.082	1.2%	0.103	0.699	3.6%	0.005	0.032	2.2%

* assumes 7 day half-life, calculation based on vaccines in previous 90 days

Prediction of log(Blood Hg)*

	Beta	P-value
Autism or ASD	-0.01	0.95
Ate tuna (1+ servings/wk)	0.56	0.001
Ate ocean fish (")	0.68	<0.0001
Ate freshwater fish (")	0.57	0.01
Hg amalgams x chew/grind teeth	0.15	0.004
Nasal spray or ear wax removal	0.52	0.02

* Multivariate analysis to adjust for confounding
  Subjects weighted to adjust for differential participation by SES

Baby Locks (first haircut)

QuickTime™ and a
TIFF (Uncompressed) decompressor
are needed to see this picture.

DISCUSSION

Discussion

- *Timing:* Current concentrations of blood metals in children 2-5 years of age unlikely to represent causal factors:
  possibly concommitant, or downstream of ASD,
- Some authors suggested abnormal metabolism or faulty excretion of metals in children with autism
- Chelation
- Statistical analyses validate use of questionnaire data about recent exposures to predict current blood Hg level

Future Work on Metals

- Earlier time windows for causal factors:
  - Analyses of baby locks (first haircuts) currently underway
  - Newborn blood spots
  - Maternal hair (if long enough)
- Evaluate subsets: early onset/regressive
- Gene x environment interaction: GSTM1, GSTT1
- Develop a pharmacokinetic model of life-course Hg profiles (need funding!)
- Analysis of other metals: Pb, Cd, As, Mn

Altered Immune Responses in Children with Autism Spectrum Disorder

Be in *CHARGE* !

http://beincharge.ucdavis.edu/
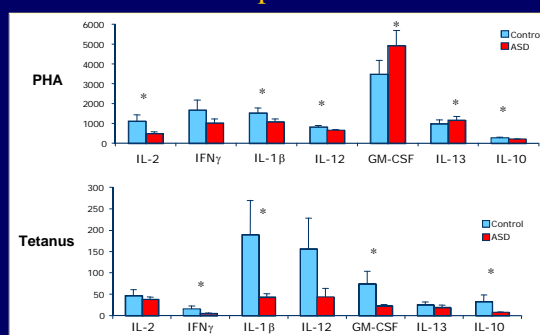
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Comparison of CHARGE
Subjects to the Target Population

	CHARGE AU (n=341)	AU Pool (n=876)	CHARGE DD (n=54)	DD Pool (n=299)	CHARGE GP (n=101)	GP Pool (n=1240)
	%	%	%	%	%	%
Male sex of child	88.0	83.3	66.7	63.9	83.2	79.4
Parents' Race: White	81.4	66.9	72.2	68.4	78.0	72.2
Black	2.4	5.3	11.1	10.1	6.0	7.6
Asian	5.9	14.2	0.0	6.1	2.0	10.2
Other	0.0	0.2	3.7	1.0	1.0	0.7
Mixed	10.3	13.4	13.0	14.5	13.0	9.3
One or Both Parents Hispanic	33.8	27.5	42.6	34.7	40.6	50.3
Non-Singletons	6.2	5.8	0	5.0	3.0	1.6
Primiparous	42.8	39.0	38.9	37.1	40.6	37.3
Mother's age ≥35 Years (at Birth of Child)	25.5	23.3	18.5	23.6	28.7	16.0
MotherÕs Education <12 yrs	6.8	9.6	14.8	21.1	12.1	29.8
16 Years or More	41.8	32.9	27.8	22.8	41.4	23.1
Mother born: Inside USA	72.4	69.1	68.5	73.2	70.3	54.5
Mexico	10.3	9.3	25.9	14.7	14.9	24.1
Outside USA or Mexico	17.3	21.7	5.6	12.0	14.9	21.4
Payment Method for Delivery						
Public	17.3	21.1	37.0	40.3	19.8	42.1
Private	81.2	77.5	63.0	57.4	80.2	56.0
Other	1.5	1.4	0.0	2.4	0.0	1.9